P1 1081466

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office

October 21, 2003

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: *60/410,778*
FILING DATE: *September 13, 2002*
RELATED PCT APPLICATION NUMBER: *PCT/US03/28742*

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

N. Woodson

N. WOODSON
Certifying Officer

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

09-16-02

A/Prov &

JC845 U.S. PTO 09/13/02

Please type a plus sign (+) inside this box → [+]

PTO/SB/16 (8-00)
Approved for use through10/31/2002 OMB 0651-0032
U S Patent and Trademark Office, U S DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

11040 U.S. PTO 60/410178

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Myung-Hee	Park	New Brunswick, NJ
Edith C.	Wolff	New Brunswick, NJ
Paul M.J.	Clement	New Brunswick, NJ
Debra	Heller	New Brunswick, NJ

☒ *Additional inventors are being named on the* 1 *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (280 characters max)

METHODS OF DIAGNOSING AND TREATING HYPERPROLIFERATIVE DISORDERS

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

☒ Customer Number 26259 →

OR *Type Customer Number here*

Place Customer Number Bar Code Label here
26259
PATENT TRADEMARK OFFICE

☐ Firm or Individual Name					
Address					
Address					
City		State		ZIP	
Country		Telephone		Fax	

ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification *Number of Pages*	24	☐ CD(s), Number	
☐ Drawing(s) *Number of Sheets*		☒ Other (specify)	Return Postcard
☐ Application Data Sheet. See 37 CFR 1.76			

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT *(check one)*

☒	Applicant claims small entity status. See 37 CFR 1.27.	FILING FEE AMOUNT ($)
☐	A check or money order is enclosed to cover the filing fees	
☒	The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number 50-1619	$80.00
☐	Payment by credit card. Form PTO-2038 is attached.	

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☐ No.

☒ Yes, the name of the U S Government agency and the Government contract number are _____

National Institute of Health Grant No.

Respectfully submitted,

SIGNATURE Jane Massey Licata

TYPED or PRINTED NAME Jane Massey Licata

TELEPHONE 856-810-1515

Date 9/13/02

REGISTRATION NO. *(if appropriate)* 32,257

Docket Number: UMD-0007

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, D.C. 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, D.C.

P19SMALL/REV05

PROVISIONAL APPLICATION COVER SHEET
Additional Page

PTO/SB/16 (8-00)
Approved for use through 10/31/2002 OMB 0651-0032
U S Patent and Trademark Office, U.S DEPARTMENT OF COMMERCE

Docket Number	UMD-0007	Type a plus sign (+) inside this box →	+

INVENTOR(S)/APPLICANT(S)		
Given Name (first and middle [if any])	Family or Surname	Residence (City and either State or Foreign Country)
Hartmut M.	Hanauske-Abel	New Brunswick, NJ
Bernadette M.	Cracchiolo	New Brunswick, NJ

Number 2 of 2

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

UMD-0007

CERTIFICATE OF EXPRESS MAILING

"Express Mail" Label No. **EV225670599US**
Date of Deposit **September 13, 2002**

I hereby certify that this paper is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R 1.10 on the date indicated above and is addressed to the "Box PROVISIONAL, P.O. Box 2327", U.S. Patent & Trademark Office, Arlington, VA 22202.

1) Patent Application Transmittal Letter (2 copies);

2) Application consisting of 24 pages of Specification, including one (1) page of Claims and one (1) page of Abstract;

3) Return Post Card; and

4) Authorization to charge deposit account in the amount of $80.00.

Jane Massey Licata
JANE MASSEY LICATA

METHODS OF DIAGNOSING AND TREATING HYPERPROLIFERATIVE DISORDERS

Introduction

This invention was made in the course of research sponsored by the National Institutes of Health. The U.S. government may have certain rights in this invention.

Background of the Invention

Normal tissue homeostasis is achieved by an intricate balance between the rate of cell proliferation and cell death. Disruption of this balance either by increasing the rate of cell proliferation or decreasing the rate of cell death can result in the abnormal growth of cells and is a major event in the development of cancer.

Cell proliferation involves many cellular processes including transcription and translation of proteins. Steady-state mRNA levels are maintained by a balance between recruitment to ribosomes for translation and degradation by nucleolytic enzymes. Eukaryotic initiation factor 5A (eIF-5A) has been implicated in several steps of RNA metabolism including both translation and mRNA degradation.

EIF-5A is a highly conserved protein encoded in the genomes of eukaryotes and archaebacteria [Chen and Liu (1997) *Biol. Signals* **6**:105-109]. Yeast and mammalian eIF5A proteins are 63% identical, indicating the importance of this protein in basic cellular processes [Schnier, et al. (1991) *Mol. Cell. Biol.* **11**:3105-3114]. Originally purified from ribosomes of rabbit reticulocyte lysates [Kemper, et al. (1976) *J. Biol. Chem.* **251**:5551-5557], eIF-5A was

described as a translation initiation factor due to its ability to stimulate the synthesis of methionyl-puromycin *in vitro* [Benne and Hershey (1978) *J. Biol. Chem.* **253**:3078-3087; Park, et al. (1993) *Biofactors* **4**:95-104). However, depletion of this factor in yeast caused only a small (30%) reduction in the protein synthesis rate [Kang and Hershey (1994) *J. Biol. Chem.* **269**:3934-3940].

Alternatively, eIF-5A may be involved in the translation of a specific subset of mRNAs, for example, those involved in the cell cycle progression [G1/S transition; Park, et al. (1993) *Biofactors* **4**:95-104; Park, et al. (1997) *Biol. Signals* **6**:115-123]. Expression of eIF-5A has also been correlated with cell proliferation: an increase in G1-arrested cells is observed after depletion of this factor in yeast [Kang and Hershey (1994) *J. Biol. Chem.* **269**:3934-3940). eIF-5A expression is induced in activated human T lymphocytes [Bevec, et al. (1994) *Proc. Natl. Acad. Sci. USA* **91**:10829-10833].

The N-terminal acetylated serine residue of eIF-5A is phosphorylated [Kang, et al. (1993) *J. Biol. Chem.* **268**:14750-14756; Klier, et al. (1993) *FEBS Lett.* **334**:360-364]; however, phosphorylation is not essential for eIF-5A function *in vivo*. A second modification to eIF-5A is hypusination of a specific lysine [Chen and Liu (1997) *Biol. Signals* **6**:105-109; Park, et al. (1997) *Biol. Signals* **6**:115-123]. Hypusine is formed through a spermidine-dependent posttranslational modification of lysine catalyzed by deoxyhypusine synthase and deoxyhypusine hydroxylase. To date, EIF-5A is the only eukaryotic protein in nature known to be hypusinated. Hypusination of eIF-5A is essential in yeast. Strains in which hypusination is blocked, by mutation of the target lysine (K51R) or deletion of the deoxyhypusine synthase gene, are inviable

[Schnier, et al. (1991) *Mol. Cell. Biol.* **11**:3105-3114; Sasaki, et al. (1996) *FEBS Lett.* **384**:151-154; Park, et al. (1998) *J. Biol. Chem.* **273**:1677-1683]. Similarly, inhibitors of hypusination block proliferation in mammalian cell lines [Hanauske-Abel, et al. (1994) *Biochim. Biophys. Acta* **1221**:115-124; Clement, et al. (2002) *Int. J. Cancer* **100**:491-498; Nishimura, et al. (2002) *Biochem. J.* **363**:761-768; Park, et al. (1994) *J. Biol. Chem.* **269**:27827-27832; Chen, et al. (1996) *Cancer Lett.* **105**:233-239; Shi, et al. (1996) *Biochim. Biophys. Acta* **1310**:119-126]. Moreover, mRNAs encoding enzymes critical for proliferation, disappear from and reappear at polysomes in concert with inhibition and disinhibition of the hypusine-forming deoxyhypusyl hydroxylase [Hanauske-Abel, et al. (1995) *FEBS Lett.* **366**:92-98].

eIF-5A may participate in the nucleocytoplasmic trafficking of the HIV-1 Rev protein/RRE complex [Ruhl, et al. (1993) *J. Cell Biol.* **123**:1309-1320; Bevec, et al. (1996) *Science* **271**:1858-1860; Bevec and Hauber (1997) *Biol. Signals* **6**:124-133; Liu, et al. (1997) *Biol. Signals* **6**:166-174; Hofmann, et al. (2001) *J. Cell Biol.* **152**:895-910]; however, eIF-5A does not directly interact with REV in a nuclear export signal-dependent manner [Henderson and Percipalle (1997) *J. Mol. Biol.* **274**:693-707] Moreover, the cellular localization of eIF-5A is not consistent with a Rev/eIF-5A interaction [Shi, et al. (1997) *Biol. Signals* **6**:143-149]. In mammalian cells, eIF-5A is mainly cytoplasmic with a fraction associated with the endoplasmic reticulum (ER) membrane through ribosomes [Shi, et al. (1996) *Exp. Cell Res.* **225**:348-356]. Its cytoplasmic localization, combined with its interaction with the ribosomal protein L5 [Schatz, et al. (1998) *Proc. Natl.*

Acad. Sci. USA **95**:1607-1612], further indicates a role for eIF-5A in translation.

A yeast mutant harboring a temperature-sensitive allele of eIF-5A, tif51A, exhibits a defect in mRNA decay, accumulating uncapped mRNAs at the restrictive temperature. In addition, this strain shows an 30% decrease in protein synthesis at high temperature [Zuk and Jacobson (1998) *EMBO J.* **17**:2914-2925]. Furthermore, poly(A)-binding protein and protein kinase C are multicopy suppressors of tif51A-1, indicating a role for eIF-5A in RNA metabolism, including translation, mRNA decay, and ribosome biogenesis [Valentini, et al. (2002) *Genetics* **160**:393-405].

The effects of hyperproliferative disorders such as cancer are catastrophic. Cancer causes over half a million deaths per year in the United States alone. Conventional strategies for the treatment of cancer include chemotherapy, radiotherapy, surgery or combinations thereof, however further advances in these strategies are limited by lack of specificity and excessive toxicity to normal tissues. Generally, both standard chemotherapy and radiotherapy, as well as transfer of genetic material into cells, have limitations; there clearly remains a need for improved strategies of anti-cancer and anti-proliferative cell therapy.

In particular, there is a need to decrease the level of cell proliferation beyond that provided by traditional therapies.

Summary of the Invention

The present invention provides a composition comprising an antibody which specifically binds to eukaryotic translation initiation factor 5A (eIF-5A).

Methods for diagnosing and treating a hyperproliferative disorder using such an antibody are provided.

The invention further provides methods of identifying agents which bind eIF-5A. The method comprises contacting eIF-5A with an agent and determining whether said agent displaces the binding of an anti-eIF-5A antibody to eIF-5A. Methods of using such agents to treat hyperproliferative disorders are also provided.

These and other aspects of the present invention are set forth in more detail in the following description of the invention.

Detailed Description of the Invention

The invention provides antibodies specific for eIF-5A. Mature eIF-5A was used to immunize rabbits to generate anti-eIF-5A antibodies using standard methodologies. One antibody, designated 'NIH 353', binds the hydroxylated (mature) form of eIF-5A. Specifically, this 'NIH 353 binds the hypusine-containing domain of eIF-5A; however, 'NIH 353' does not bind to the same domain containing either lysine or deoxyhypusine, the biochemical precursors of hypusine. Immunocytochemistry analysis revealed that proliferating cells readily bound 'NIH 353' antibody. Formalin-fixed, paraffin-embedded human tonsils were sectioned such that they contained two proliferative areas, the germinal centers of lympoid follicles and the basal layer of the squamous epithelium. Following a well-known optimal antigen retrieval protocol involving microwave irradiation, the sections were contacted with 'NIH 353', and binding of 'NIH 353' to eIF-5A was detected using standard streptavidin-biotin/horseradish peroxidase methodologies with diamniobenzidine as the chromogen and hematoxylin as the counterstain. The cytoplasm of cells

only in the basal layer of the epithelium and in the germinal centers stained prominently. Nuclei remained unlabelled. For comparison, these same tissues were then reacted with antibodies against Ki67 nuclear antigen, a known proliferative marker. Similar results were obtained when 'NIH 353' was reacted with endometrium and stratified epithelium mucosa.

Antibodies or antibody fragments of the invention which are specific for mature eIF-5A may be natural or partially or wholly synthetically produced. All derivatives thereof which maintain specific eIF-5A binding ability are also included. The antibodies may be monoclonal or polyclonal and may be a member of any immunoglobulin class, including any of the human classes: IgG, IgM, IgA, IgD, and IgE. Derivatives of the IgG class, however, are preferred in the present invention.

eIF-5A antibody fragments may be any derivative of an antibody which is less than full-length. Preferably, the antibody fragment retains at least a significant portion of the full-length antibody's specific binding ability. Examples of antibody fragments include, but are not limited to, Fab, Fab', $F(ab')_2$, scFv, Fv, dsFv diabody, or Fd fragments. The antibody fragment may be produced by any means. For instance, the antibody fragment may be enzymatically or chemically produced by fragmentation of an intact antibody or it may be recombinantly produced from a gene encoding the partial antibody sequence. As used herein, antibody also includes bispecific and chimeric antibodies.

Naturally produced monoclonal antibodies may be generated using classical cloning and cell fusion techniques. In general, mature eIF-5A is administered (*e.g.*, intraperitoneal injection) to wild-type or inbred

mice (*e.g.*, BALB/c) or transgenic mice which produce desired antibodies, or rats, rabbits, chickens, sheep, goats, or other animal species which can produce native or human antibodies. The mature eIF-5A may be administered alone, or mixed with adjuvant. After the animal is boosted, for example, two or more times, the spleen or large lymph node, such as the popliteal in rat, is removed and splenocytes or lymphocytes are extracted and fused with myeloma cells using well-known processes, for example Kohler and Milstein [(1975) Nature **256**:495-497] and Harlow and Lane [Antibodies: A Laboratory Manual (Cold Spring Harbor Laboratory, New York (1988)]. The resulting hybrid cells are then cloned in the conventional manner, *e.g.* using limiting dilution, and the resulting clones, which produce the desired monoclonal antibodies, and are cultured.

Alternatively, eIF-5A antibodies are derived by a phage display method. Methods of producing phage display antibodies are well-known in the art, for example, Huse, et al. [(1989) *Science* **246**(4935):1275-81].

Selection of eIF-5A-specific antibodies is based on binding affinity to eIF-5A and may be determined by various well-known immunoassays including, enzyme-linked immunosorbent, chemiluminescent, immunofluorescent, immunohistochemical, radioimmunoassay, and immunoprecipitation assays and the like which may be performed *in vitro*, *in vivo* or *in situ*. The standard techniques known in the art for immunoassays are described in "Methods in Immunodiagnosis", 2nd Edition, Rose and Bigazzi, eds. John Wiley & Sons, 1980; Campbell et al., "Methods and Immunology", W.A. Benjamin, Inc., 1964; and Oellerich, M. (1984) *J. Clin. Chem. Clin. Biochem.* **22**:895-904.

One aspect of the invention provides a method of using an eIF-5A antibody to diagnose a hyperproliferative disorder in a subject. As eIF-5A is essential for cell proliferation, it provides a general biomarker for hyperproliferative disorders in which cell growth is independent of normal regulatory mechanisms (*e.g.*, loss of contact inhibition). This includes, but is not limited to, the abnormal growth of tumor cells, both benign and malignant, due to direct expression of an oncogene or as a result of oncogenic mutation in another gene, or as a result of aberrant cell cycle regulation. Thus, compositions and methods provided herein are particularly deemed useful for the diagnosis of hyperproliferative disorders including solid tumors such as skin, breast, brain, cervical carcinomas, testicular carcinomas, etc. More particularly, cancers that may be treated by the compositions and methods of the invention include, but are not limited to, Cardiac: sarcoma (angiosarcoma, fibrosarcoma, rhabdomyosarcoma, liposarcoma), myxoma, rhabdomyoma, fibroma, lipoma and teratoma; Lung: bronchogenic carcinoma [squamous cell, undifferentiated small cell, undifferentiated large cell, adenocarcinoma], alveolar [bronchiolar] carcinoma, bronchial adenoma, sarcoma, lymphoma, chondromatous hamartoma, mesotheliorna; Gastrointestinal: esophagus [squamous cell carcinoma, adenocarcinoma, leiomyosarcoma, lymphoma], stomach [carcinoma, lymphoma, leiomyosarcoma], pancreas [ductal adenocarcinoma, insulinoma, glucagonoma, gastrinoma, carcinoid tumors, vipoma], small bowel [adenocarcinoma, lymphoma, carcinoid tumors, Karposi's sarcoma, leiomyoma, hemangioma, lipoma, neurofibroma, fibroma], large bowel [adenocarcinoma, tubular adenoma, villous adenoma, hamartoma, leiomyoma]; Genitourinary tract: kidney

[adenocarcinoma, Wilm's tumor (nephroblastoma), lymphoma, leukemia], bladder and urethra [squamous cell carcinoma, transitional cell carcinoma, adenocarcinoma], prostate [adenocarcinoma, sarcoma], testis [seminoma, teratoma, embryonal carcinoma, teratocarcinoma, choriocarcinoma, sarcoma, interstitial cell carcinoma, fibroma, fibroadenoma, adenomatoid tumors, lipoma]; Liver: hepatoma [hepatocellular carcinoma], cholangiocarcinorna, hepatoblastoma, angiosarcoma, hepatocellular adenoma, hemangioma; Bone: osteogenic sarcoma [osteosarcoma], fibrosarcoma, malignant fibrous histiocytoma, chondrosarcoma, Ewing's sarcoma, malignant lymphoma [reticulum cell sarcoma], multiple myeloma, malignant giant cell tumor chordoma, osteochronfroma [osteocartilaginous exostoses], benign chondroma, chondroblastoma, chondromyxofibroma, osteoid osteoma and giant cell tumors; Nervous system: skull [osteoma, hemangioma, granuloma, xanthoma, osteitis deformans], meninges [meningioma, meningiosarcoma, gliomatosis], brain [astrocytoma, medulloblastoma, glioma, ependymoma, germinoma (pinealoma), glioblastoma multiform, oligodendroglioma, schwannoma, retinoblastoma, congenital tumors], spinal cord [neurofibroma, meningioma, glioma, sarcoma]; Gynecological: uterus [endometrial carcinoma], cervix [cervical carcinoma, pre-tumor cervical dysplasia], ovaries [ovarian carcinoma (serous cystadenocarcinorna, mucinous cystadenocarcinoma, unclassified carcinoma), granulosa-thecal cell tumors, Sertoli-Leydig cell tumors, dysgerminoma, malignant teratoma], vulva [squamous cell carcinoma, intraepithelial carcinoma, adenocarcinoma, fibrosarcoma, melanoma], vagina [clear cell carcinoma, squamous cell carcinoma, botryoid sarcoma (embryonal rhabdomyosarcoma), fallopian tubes [carcinoma]; Hematologic: blood [myeloid leukemia (acute

and chronic), acute lymphoblastic leukemia, chronic lymphocytic leukemia, myeloproliferative diseases, multiple myeloma, myelodysplastic syndrome], Hodgkin's disease, non-Hodgkin's lymphoma (malignant lymphoma); Skin: malignant melanoma, basal cell carcinoma, squamous cell carcinoma, Karposi's sarcoma, moles dysplastic nevi, lipoma, angioma, dermatofibroma, keloids, psoriasis; and Adrenal glands: neuroblastoma.

The diagnostic method of the invention provides contacting a biological sample such as a biopsy sample, tissue, cell or fluid (*e.g.*, whole blood, plasma or urine) isolated from a subject with an antibody which binds eIF-5A. The antibody is allowed to bind to the eIF-5A antigen to form an antibody-antigen complex. The eIF-5A antigen, as used herein, includes the mature eIF-5A, a hypusine-containing fragment of eIF-5A, or a hypusine amino acid. The conditions and time required to form the antibody-antigen complex may vary and are dependent on the biological sample being tested and the method of detection being used. Once non-specific interactions are removed by, for example, washing the sample, the antibody-antigen complex is detected using any one of the immunoassays described above as well a number of well-known immunoassays used to detect and/or quantitate antigens [see, for example, Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, New York (1988) 555-612]. Such well-known immunoassays include antibody capture assays, antigen capture assays, and two-antibody sandwich assays. In an antibody capture assay, the antigen is attached to solid support, and labeled antibody is allowed to bind. After washing, the assay is quantitated by measuring the amount of antibody retained on the solid support. In an antigen capture assay, the antibody is

attached to a solid support, and labeled antigen is allowed to bind. The unbound proteins are removed by washing, and the assay is quantitated by measuring the amount of antigen that is bound. In a two-antibody sandwich assay, one antibody is bound to a solid support, and the antigen is allowed to bind to this first antibody. The assay is quantitated by measuring the amount of a labeled second antibody that binds to the antigen.

These immunoassays typically rely on labeled antigens, antibodies, or secondary reagents for detection. These proteins may be labeled with radioactive compounds, enzymes, biotin, or fluorochromes. Of these, radioactive labeling may be used for almost all types of assays. Enzyme-conjugated labels are particularly useful when radioactivity must be avoided or when quick results are needed. Biotin-coupled reagents usually are detected with labeled streptavidin. Streptavidin binds tightly and quickly to biotin and may be labeled with radioisotopes or enzymes. Fluorochromes, although requiring expensive equipment for their use, provide a very sensitive method of detection. Those of ordinary skill in the art will know of other suitable labels which may be employed in accordance with the present invention. The binding of these labels to antibodies or fragments thereof may be accomplished using standard techniques such as those described by Kennedy, et al. [(1976) *Clin. Chim. Acta* **70**:1-31], and Schurs, et al. [(1977) *Clin. Chim Acta* **81**:1-40].

In accordance with the diagnostic method of the invention, the presence or absence of the antibody-antigen complex is correlated with the presence or absence of eIF-5A antigen in the biological sample. A biological sample containing eIF-5A antigen is indicative of a hyperproliferative disorder in the subject from which the

biological sample was obtained. Accordingly, the diagnostic method of the invention may be used as part of a routine screen in subjects suspected of having a hyperproliferative disorder or for subjects who may be genetically predisposed to having a hyperproliferative disorder. Moreover, the diagnostic method of the invention may be used alone or in combination with other well-known diagnostic methods to confirm a hyperproliferative disorder.

The diagnostic method of the invention further provides that an antibody of the invention may be used to monitor the levels of eIF-5A antigen in patient samples at various intervals of drug treatment to identify whether the drug treatment is effective in reducing or inhibiting hyperproliferation of cells. Furthermore, eIF-5A antigen levels may be monitored using an antibody of the invention in studies evaluating drug toxicity and efficacy of drug candidates in model systems. For example, using an anti-eIF-5A antibody, eIF-5A antigen levels may be monitored for biological samples treated with known or unknown therapeutic agents or toxins. This may be accomplished with cell lines *in vitro* or in various model systems, depending on the hyperproliferative disorder being investigated. The presence of eIF-5A antigen in biological samples treated with a drug candidate indicates that the drug candidate has little or no effect on cell proliferation. Likewise, the absence of eIF-5A antigen indicates that the drug candidate is effective in reducing or inhibiting cell proliferation. This may provide valuable information at all stages of clinical drug trials as well as subsequent monitoring of patients undergoing drug treatment.

Another aspect of the invention provides that an anti-eIF-5A antibody, or fragment thereof may be administered to

a human or other animal in an amount to decrease or inhibit cell proliferation. As one may appreciate, any hyperproliferative disorder which may be diagnosed by an antibody of the invention, may also be treated using an antibody of the invention. A skilled clinician or physician would be able, by routine experimentation, to determine what an effective, non-toxic amount of antibody would be for the purpose of decreasing or inhibiting cell proliferation. Generally, however, an effective dosage will be in the range of about 0.05 to 100 milligrams per kilogram body weight per day.

Furthermore, an antibody of the invention may be administered to a human or other animal in a conventional dosage form prepared by combining an antibody of the invention with a conventional pharmaceutically acceptable carrier or diluent according to known techniques. It will be recognized by one of skill in the art that the form and character of the pharmaceutically acceptable carrier or diluent is dictated by the amount of active ingredient with which it is to be combined, the route of administration and other well-known variables.

The route of administration of an anti-eIF-5A antibody, or fragment thereof, may be oral, parenteral, by inhalation or topical. The term parenteral as used herein includes intravenous, intramuscular, subcutaneous, rectal, vaginal or intraperitoneal administration. The subcutaneous and intramuscular forms of parenteral administration are generally preferred.

The daily parenteral and oral dosage regimens for employing antibodies of the invention to therapeutically decrease cell proliferation will generally be in the range of about 0.05 to 100, but preferably about 0.5 to 10, milligrams per kilogram body weight per day.

An antibody of the invention may also be administered by inhalation. Inhalation, as used herein, includes intranasal and oral inhalation administration. Appropriate dosage forms for such administration, such as an aerosol formulation or a metered dose inhaler, may be prepared by conventional techniques. The preferred dosage amount of an antibody of the invention to be employed is generally within the range of about 10 to 100 milligrams.

An antibody of the invention may also be administered topically. By topical administration is meant non-systemic administration and includes the application of an anti-eIF-5A antibody, or fragment thereof, externally to the epidermis, to the buccal cavity and instillation of such an antibody into the ear, eye and nose, and where it does not significantly enter the blood stream. By systemic administration is meant oral, intravenous, intraperitoneal and intramuscular administration. The amount of an antibody required for therapeutic effect will, of course, vary with the antibody chosen, the nature and severity of the condition being treated and the animal undergoing treatment, and is ultimately at the discretion of the physician. A suitable topical dose of an antibody of the invention will generally be within the range of about 1 to 100 milligrams per kilogram body weight daily.

While it is possible for an antibody or fragment thereof to be administered alone, it is preferable to present it as a pharmaceutical composition. A generally recognized compendium of methods and ingredients of pharmaceutical compositions is Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, PA. The active ingredient may comprise, for topical administration, from 0.001% to 10% w/w, *e.g.*, from 1% to 2% by weight of the composition, although it may comprise as much as 10%

w/w but preferably not in excess of 5% w/w and more preferably from 0.1% to 1% w/w of the composition.

Topical compositions of the invention, may comprise an antibody of the invention together with one or more acceptable carrier(s) and optionally any other therapeutic ingredients(s). The carrier(s) must be acceptable in the sense of being compatible with the other ingredients of the composition and not deleterious to the recipient thereof.

Compositions suitable for topical administration include liquid or semi-liquid preparations suitable for penetration through the skin to the site of where treatment is required, such as liniments, lotions, creams, ointments or pastes, and drops suitable for administration to the eye, ear or nose.

Drops may comprise sterile aqueous or oily solutions or suspensions and may be prepared by dissolving an antibody of the invention in a suitable aqueous solution of a bactericidal and/or fungicidal agent and/or any other suitable preservative, and preferably including a surface active agent. The resulting solution may then be clarified by filtration, sterilized by filtration and transferred to a container by an aseptic technique. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops are phenylmercuric nitrate or acetate (0.002%), benzalkonium chloride (0.01%) and chlorhexidine acetate (0.01%). Suitable solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.

Lotions include those suitable for application to the skin or eye. An eye lotion may comprise a sterile aqueous solution optionally containing a bactericide and may be prepared by methods similar to those for the preparation of drops. Lotions or liniments for application to the skin may

also include an agent to hasten drying and to cool the skin, such as an alcohol or acetone, and/or a moisturizer such as glycerol or an oil such as castor oil or arachis oil.

Creams, ointments or pastes according to the invention are semi-solid compositions of an anti-eIF-5A antibody or fragment thereof for external application. They may be made by mixing an antibody in finely-divided or powdered form, alone or in solution or suspension in an aqueous or non-aqueous fluid, with the aid of suitable machinery, with a greasy or non-greasy basis. The basis may comprise hydrocarbons such as hard, soft or liquid paraffin, glycerol, beeswax, a metallic soap; a mucilage; an oil of natural origin such as almond, corn, arachis, castor or olive oil; wool fat or its derivatives, or a fatty acid such as stearic or oleic acid together with an alcohol such as propylene glycol or macrogels. The composition may incorporate any suitable surface active agent such as an anionic, cationic or non-ionic surface active such as sorbitan esters or polyoxyethylene derivatives thereof. Suspending agents such as natural gums, cellulose derivatives or inorganic materials such as silicaceous silicas, and other ingredients such as lanolin, may also be included.

The antibodies and pharmaceutical compositions of the invention are particularly useful for parenteral administration, *i.e.*, subcutaneously, intramuscularly or intravenously. The compositions for parenteral administration will commonly comprise a solution of an antibody or fragment thereof of the invention or a cocktail thereof dissolved in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be employed, *e.g.*, water, buffered water, 0.4% saline, 0.3%

glycine, and the like. These solutions are sterile and generally free of particulate matter. These solutions may be sterilized by conventional, well-known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, etc. The concentration of the antibody or fragment thereof of the invention in such pharmaceutical compositions may vary widely, *i.e.*, from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight, and will be selected primarily based on fluid volumes, viscosities, etc., according to the particular mode of administration selected.

Thus, a pharmaceutical composition of the invention for intramuscular injection could be prepared to contain 1 ml sterile buffered water, and 50 mg of an antibody or fragment thereof of the invention. Similarly, a pharmaceutical composition of the invention for intravenous infusion could be made up to contain 250 ml of sterile Ringer's solution, and 150 mg of an antibody or fragment thereof of the invention. Actual methods for preparing parenterally administrable compositions are well-known or will be apparent to those skilled in the art, and are described in more detail in, *e.g.*, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, PA.

The antibodies, or fragments thereof, of the invention may be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique is effective with conventional immunoglobulins and art-known lyophilization and reconstitution techniques may be employed.

The pharmaceutical composition of the invention may be administered for prophylactic and/or therapeutic treatments. In therapeutic application, compositions are administered to a subject already suffering from a hyperproliferative disorder, in an amount sufficient to cure or at least partially arrest the disorder and its complications. In prophylactic applications, compositions containing the present antibodies or fragments thereof are administered to a subject not already in a disease state but one that may be predisposed to a hyperprolifertive disorder to enhance the subject's resistance.

It will be recognized by one of skill in the art that the optimal quantity and spacing of individual dosages of an antibody or fragment thereof of the invention will be determined by the nature and extent of the hyperproliferative disorder being treated, the form, route and site of administration, and the particular animal being treated, and that such optimums may be determined by conventional techniques. It will also be appreciated by one of skill in the art that the optimal course of treatment, *i.e.*, the number of doses of an antibody or fragment thereof of the invention given per day for a defined number of days, may be ascertained by those skilled in the art using conventional course of treatment determination tests.

A still further aspect of the invention relates to screening assays to identify agents which inhibit or displace the binding of an anti-eIF-5A antibody to eIF-5A. The invention encompasses both *in vivo* and *in vitro* assays to screen small molecules, compounds, recombinant proteins, peptides, nucleic acids, antibodies, etc. which bind mature eIF-5A and thus, identify potential therapeutic agents for the treatment of hyperproliferative disorders.

In a preferred embodiment, the binding of the agent is determined through the use of competitive binding assays. The competitor is an antibody of the invention known to bind to the eIF-5A protein. Competitive screening assays may be done by combining the eIF-5A protein and an agent in a first sample. A second sample comprises an agent, eIF-5A protein and an antibody of the invention. The binding of the agent is determined for both samples, and a change, or difference in binding between the two samples indicates the presence of an agent capable of binding to eIF-5A protein and potentially modulating its activity. That is, if the binding of the agent is different in the second sample relative to the first sample, the agent is capable of binding to eIF-5A protein.

One variation provides that the agent is labeled. Either the agent, or the competitor, or both, is added first to eIF-5A protein for a time sufficient to allow binding. Incubations may be performed at any temperature which facilitates optimal activity, typically between 4 and 40°C. Incubation periods are selected for optimum activity, but may also be optimized to facilitate rapid high-throughput screening. Typically between 0.1 and 1 hour will be sufficient. Excess reagent is generally removed or washed away. The second component is then added, and the presence or absence of the labeled component is followed, to indicate binding.

It is preferred that the competitor is added first, followed by the agent. Displacement of the competitor is an indication that the agent is binding to eIF-5A protein and thus is capable of binding to, and potentially modulating, the activity of eIF-5A protein. In this reaction either component may be labeled. Thus, for example, if the competitor is labeled, the presence of label in the wash

solution indicates displacement by the agent. Alternatively, if the agent is labeled, the presence of the label on the support indicates displacement.

Alternatively, the agent is added first, with incubation and washing, followed by the competitor. The absence of binding by the competitor may indicate the agent is bound to eIF-5A protein with a higher affinity. Thus, if the agent is labeled, the presence of the label on the support, coupled with a lack of competitor binding, may indicate the agent is capable of binding to eIF-5A protein.

Agents encompass numerous chemical classes, though typically they are organic molecules, preferably small organic compounds having a molecular weight of more than 100 and less than about 2,500 daltons. Agents comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The agents often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Agents may also be found among biomolecules including peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof.

Agents are obtained from a wide variety of sources including libraries of synthetic or natural compounds.

Alternatively, the antibodies of this invention may be used for the design and synthesis of either peptide or non-peptide compounds (mimetics) specific to eIF-5A [see, *e.g.*, Saragovi, et al (1991) *Science* **253**:792-795].

The assays provided use eIF-5A protein. Alternatively, portions of the eIF-5A protein may be used. For example,

the region of eIF-5A which is homologous to dihydrofolate reductase and contains the hypusine residue may be used or the region homologous to cold-shock protein A may be used. In addition, the assays described herein may use either isolated eIF-5A protein or cells or animal models comprising the eIF-5A protein.

A variety of other reagents may be included in the screening assays. These include reagents like salts, neutral proteins, *e.g.* albumin, detergents, etc. which may be used to facilitate optimal protein-protein binding and/or reduce non-specific or background interactions. Also, reagents that otherwise improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, and the like may be used. The mixture of components may be added in any order that provides for the requisite binding.

The methods of the invention are used to identify compounds which inhibit or displace an antibody which binds to eIF-5A and are therefore useful in the treatment of hyperproliferation disorders. Hyperproliferative disorders which can be treated by the methods and compositions provided herein include, but are not limited to, cancers (as described above), autoimmune disease, arthritis, graft rejection, inflammatory bowel disease, proliferation induced after medical procedures, including, but not limited to, surgery, angioplasty, and the like.

In the same manner that an antibody of the invention may be administered to treat a hyperproliferative disorder, so may an agent that inhibits or displaces an anti-eIF-5A antibody be administered to treat a hyperproliferative disorder. Accordingly, a further aspect of the invention provides methods for decreasing cell proliferation by providing to a subject with a hyperproliferative disorder

an agent which displaces or inhibits the binding of an anti-eIF-5A antibody to eIF-5A. As one of skill in the art may appreciate, the pharmaceutical compositions comprising the agent and a pharmaceutically acceptable carrier, as well as the route of administration of such pharmaceutical compositions, would be similar to those provided above for an anti-eIF-5A antibody. The optimal quantity and spacing of individual dosages of an agent of the invention will be determined by the nature of the agent, the nature and extent of the hyperproliferative disorder being treated, the form, route and site of administration, and the particular animal being treated. Such optimums may be determined by conventional techniques of monitoring cell proliferation.

What is claimed is:

1. An antibody specific for eukaryotic initiation factor 5A (eIF-5A).

2. The antibody of claim 1, wherein the antibody specifically binds a hypusine-containing fragment of eIF-5A.

3. A method of diagnosing a hyperproliferative disorder comprising contacting a biological sample with an antibody of claim 1 and detecting said antibody bound to eIF-5A present in the sample which is indicative of a hyperproliferative disorder.

4. A method of treating a hyperproliferative disorder comprising administering an effective amount of an antibody of claim 1 to a subject so that hyperproliferation is inhibited.

5. A method of identifying an agent that decreases cell proliferation comprising contacting eIF-5A with an agent and detecting the binding of an antibody of claim 1 to eIF-5A.

6. A method of treating a hyperproliferative disorder comprising administering an effective amount of an agent which displaces the binding of an antibody of claim 1 to eIF-5A so that hyperproliferation is inhibited.

Abstract

The invention relates to compositions and methods for diagnosing and treating hyperproliferative disorders using an antibody which specifically recognizes eukaryotic translation initiation factor 5A (eIF-5A). The invention further relates to methods of identifying agents which displace the binding of an anti-eIF-5A antibody to eIF-5A. Such agents are useful for treating hyperproliferative disorders.